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# Epidemiology of Nosocomial Acquisition of *Candida lusitaniae*

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***Candida* species are important nosocomial pathogens; however, little is known about the epidemiology of *Candida lusitaniae*, an organism frequently resistant to amphotericin B. We evaluated 98 patients admitted to the bone marrow transplant and medical intensive care units of a tertiary-care hospital. Each patient with *C. lusitaniae* was matched with control patients. Restriction fragment analysis of DNA was used to determine strain relatedness. Seven patients (7.1%) with *C. lusitaniae* were identified; five acquired *C. lusitaniae* after admission to the study unit. All isolates were susceptible to amphotericin B. There were no differences between patients and controls with regard to duration of stay in the study unit, antibiotic administration, antifungal therapy, immunosuppressive therapy, catheter use, or underlying disease. Temporal and geographic clustering of five patients with identical strains occurred. No common source was identified. Restriction fragment analysis revealed a total of eight strains, and five patients shared one strain type. These results demonstrate exogenous acquisition of *C. lusitaniae*. The mechanism of acquisition is probably indirect contact transmission between patients.**

*Candida lusitaniae* was originally isolated from the intestinal contents of warm-blooded animals (25). In humans, *C. lusitaniae* rarely causes opportunistic infections, although 13 cases involving various sites, including the kidneys, peritoneum, and blood stream, have been described (2, 5, 8–10, 12, 18, 23). This species of *Candida* is of special interest because of its innate resistance to amphotericin B and its ability to develop resistance to amphotericin during therapy (1, 2, 8, 9, 15). Little is known about the epidemiology of infection with *C. lusitaniae*. Studies suggest that broad-spectrum antibiotics and prolonged hospitalization may contribute to both colonization and infection (2, 5, 9, 10, 12, 13, 18). To evaluate the epidemiology of nosocomial infection, we conducted a prospective study to identify risk factors, patient and hospital reservoirs, and possible mechanisms of transmission.

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Harper Hospital is a 900-bed teaching hospital in Detroit, Mich. The medical intensive care unit (ICU) is an eight-bed private-room unit. Patients in the ICU were transferred halfway through the study period to a separate 10-bed unit with four open beds and six private beds on a different floor. The bone marrow transplant unit (BMTU) is a 24-bed private-room unit with a staff separate from that of the ICU. Patients are occasionally transferred between the BMTU and ICU, depending on severity of illness. We prospectively studied all patients who gave informed consent and were hospitalized in the BMTU or ICU during the 7.5-month study period. Demographic and clinical data, including date of hospitalization, age, gender, ward, and medical service, were recorded. Presence of urinary and vascular catheters, movement within the hospital, exposure to antibiotics, and cytotoxic and immunosuppressive agents were recorded. We defined colonization and infection according to established criteria: colonization was indicated when *Candida* species were isolated from a site without signs or symptoms of infection; and infection was indicated when the organism was isolated from a normally sterile body site, concurrent with signs and symptoms of infection. Death was considered related to *Candida* species if the patient died within 72 h after a positive culture had been obtained from a sterile site and the clinical profile was consistent with septicemia.

Specimens were obtained when patients were admitted to the study unit, weekly for patients in the BMTU and biweekly for patients in the ICU until discharge. The dates, sites, and colony counts of cultures positive for yeast were recorded. Urine samples were obtained through clean-catch voiding or were taken from syringe-aspirated catheters. An environmental culture survey was performed in every patient room and included at least one culture of surfaces in contact with hands or patients, ventilator tubing, nebulizer, humidifier, floor, and sink area. Hand cultures of 25 hospital personnel were obtained by producing an impression on a petri dish with Sabouraud dextrose agar. Cultures of environmental surfaces from the study units were done by using Rodac plates (Falcon Inc., Oxnard, Calif.). Cultures of surfaces and hands were performed at 2-month intervals during the study period. Surfaces in one ICU were also cultured before patients were admitted to it when an ICU location was moved.

Patients with *C. lusitaniae* were matched by time of admission to the unit with three control patients who did not develop *Candida* species colonization. Statistical significance between patients and controls was evaluated by using tests of homogeneity of proportions and bivariate analysis for dichotomous outcome data. Continuous variables were compared with the Mann-Whitney U test. A multivariate linear-regression model was used to evaluate independence of risk factors.

All *C. lusitaniae* isolates were identified by negative germ tube formation, morphology, and the yeast API 20C method

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TABLE 1. Recovery of *C. lusitaniae* by source of sample

| Source | Total no. of *C. lusitaniae* isolates | % of *Candida* isolates | % Positive for *C. lusitaniae* |
|---|---|---|---|
| Oropharynx | 3 | 119 | 2.5 |
| Stool | 13 | 138 | 9.4 |
| Urine | 8 | 77 | 10.4 |
| Vagina | 5 | 36 | 13.9 |
| Blood | 0 | 4 | 0 |
| Wound | 0 | 14 | 0 |
| Sputum | 0 | 2 | 0 |
| Total | 29[a] | 390 | 7.4 |

[a] Seventeen isolates were from one patient.

(Sherwood Medical, Plainview, N.Y.). The results were verified by carbohydrate assimilation and fermentation tests. Control strains of *C. lusitaniae* were obtained from two patients located in a geographically separate hospital, and *C. lusitaniae* ATCC 34449 was also used as a control strain. Susceptibility of *C. lusitaniae* isolates to amphotericin B was evaluated by using previously published methods (6). Isolates were evaluated for molecular relatedness by using restriction enzyme analysis (REA). These methods have been previously used to evaluate the relatedness of bacteria, viruses, and yeasts (7, 14, 19, 20, 26, 28). For the isolation of DNA, yeast cells were grown on Sabouraud dextrose agar plates at 30°C for 36 h. The method used to isolate genomic DNA has been previously published (26). For the electrophoresis of samples, 20 μg of DNA was digested with 5 μl of the restriction endonucleases *Eco*RI and *Msp*I (Bethesda Research Laboratories, Gaithersburg, Md.), following the recommendations of the manufacturer. The DNA was run on a 0.7% agarose gel apparatus at 30 V for 16 h. The gel was stained in a solution of 0.5 μg of ethidium bromide per ml and then destained in water before being photographed.

During the study period, we monitored 98 patients hospitalized in the BMTU and ICU. Six patients were transferred between the BMTU and the ICU during their hospital stays. Patients were hospitalized in the ICU for a mean of 11.3 days (range, 2 to 43 days) and in the BMTU for a mean of 27.4 days (range, 3 to 73 days). Seven patients were found to harbor *C. lusitaniae* during their hospital stay. One patient with *C. lusitaniae* was readmitted; two were transferred between the BMTU and the ICU. The recovery of *C. lusitaniae* by sample source and as a proportion of total *Candida* species is shown in Table 1. The hospital location of patients with *C. lusitaniae* is shown in Fig. 1 and Table 2. We recovered a total of 29 isolates of *C. lusitaniae* from the seven different patients. All isolates were susceptible to amphotericin B in vitro, with a MIC for 90% of strains tested of 0.4 μg/ml (range, <0.05 to 0.4 μg/ml). Evaluation of longitudinal cultures showed that resistance to amphotericin B did not develop. Initial cultures from two patients (length of catheterization, 4 days and 12 weeks) grew colonies of *C. lusitaniae* from multiple sources, and these sources (urine, stool, oropharynx, and vagina) remained positive during the study period. One of these patients was in the ICU and was transferred to the BMTU. Both patients with initial cultures positive for *C. lusitaniae* had been hospitalized in a nonstudy unit for at least 72 h prior to transfer to the study unit, and both had been transferred to the hospital from different extended-care facilities, a community nursing home and a rehabilitation center. Five patients had negative initial cultures and grew *C. lusitaniae* after admission to the study unit. Four of five of these patients were located in the BMTU. Initial sources of isolates in patients who acquired *C. lusitaniae* while in hospital were the rectum for four and the urine for one patient without a prior history of urinary bladder catheterization. The patient with *C. lusitaniae* in the urine developed a symptomatic urinary tract infection secondary to the organism. Treatment with amphotericin B bladder irrigations was performed, and subsequent urine cultures showed eradication of the organism. None of the five patients who became colonized with *C. lusitaniae* while in the hospital developed infection at the site of isolation. Both patients with initial cultures positive for *C. lusitaniae* expired, but deaths were not considered related to *C. lusitaniae* infections. There were no deaths among patients with nosocomial acquisition of *C. lusitaniae*.

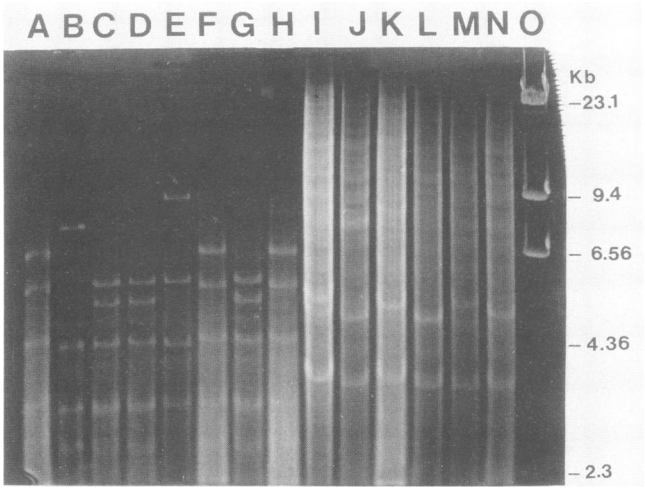


FIG. 1. Agarose gel electrophoresis of purified genomic DNA from eight separate patient isolates of *C. lusitaniae*. Lanes: A to H, digested by *Msp*I; I to N, digested by *Eco*RI and correspond to isolates in lanes A, F, H, C, D, and G, respectively. Lane O, bacteriophage lambda digested with *Eco*RI. The positions of markers (in kilobases) are indicated on the right.

Risk factors and demographic data for patients with *C. lusitaniae*, compared with those for controls, are shown in Table 3. There were no risk factors found independently associated with *C. lusitaniae*. Hand cultures of 25 personnel in the two units were all negative for *C. lusitaniae*. *C. lusitaniae* was recovered from two environmental surfaces in the ICU, but no environmental sites were culture positive in the BMTU. The environmental surfaces that were found to contain *C. lusitaniae* were those that were frequently touched by hospital personnel or in open contact with the entire unit, a cardiac monitor mounted in the central nursing

TABLE 2. Locations of patients with *C. lusitaniae*[a]

| Unit | No. of patients | | |
|---|---|---|---|
| | In unit | With new acquisition | With colonies when transferred to unit |
| ICU | 44 | 1 | 2 |
| BMTU | 54 | 4 | 0 |
| Total | 98 | 5 | 2 |

[a] Readmitted and transferred patients are not included.

TABLE 3. A comparison of demographic and risk factor characteristics for patients and controls

| Characteristic | Value for group | |
|---|---|---|
| | Patient | Control |
| Total no. of subjects | 7 | 21 |
| Mean age (range) [yr] | 50.3 (19–76) | 37.3 (5–68) ($P < 0.01$) |
| Female/male | 5/2 | 8/13 ($P < 0.005$) |
| Mean duration in unit (range) [days] | 27.8 (7–56) | 24.5 (7–46) |
| Prior antibiotic treatment | | |
| No. (%)[a] | 6 (86) | 18 (86) |
| Mean duration (range) [days] | 17.6 (0–33) | 18.5 (0–60) |
| Antifungal therapy[b] (no. [%]) | 7 (100) | 19 (90) |
| Granulocytopenia | 4 (57) | 11 (52) |
| No. (%) | 10.7 (8–12) | 17.8 (5–9) |
| Mean duration (range) [days] | | |
| Immunosuppressive therapy (no. [%]) | 4 (60) | 14 (66.6) |
| Underlying illness (no.) | | |
| Leukemia | | |
| Acute | 3 | 5 |
| Chronic | 1 | 1 |
| Lymphoma | | |
| Hodgkin's | 0 | 4 |
| Non-Hodgkin's | 0 | 3 |
| Other | 2 | 7 |
| Total (no. [%]) | 6 (86) | 20 (95) |

[a] No., number of subjects.
[b] Antifungal therapy, topical to systemic.

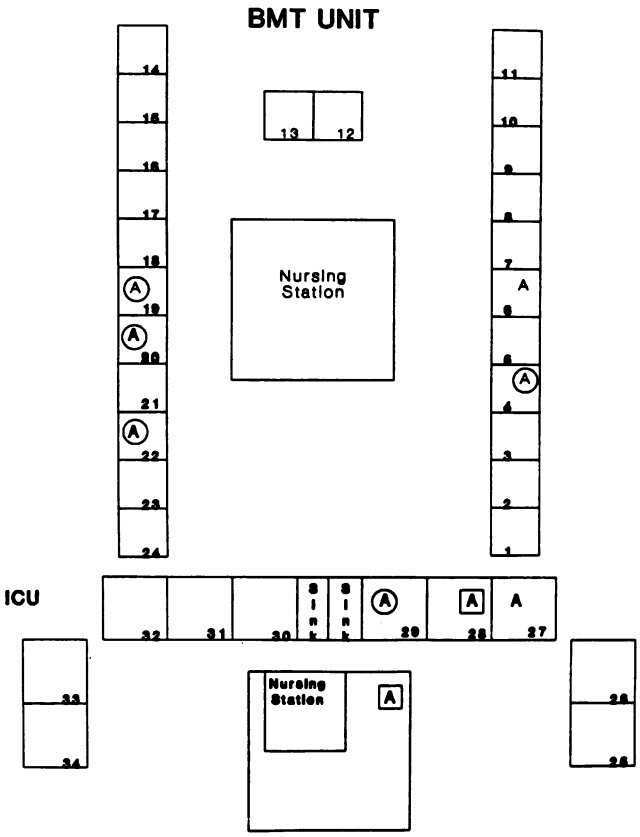


FIG. 2. Locations of cultures positive for *C. lusitaniae*. The letter A indicates identical strains of *C. lusitaniae*, which include those from transferred patients and readmitted patients (two). A denotes environmental isolates; Ⓐ denotes acquisition of strain by patient in the unit.

station and an air-conditioning/heating vent located in an open area room. No common source was identified despite careful investigation. Strains from environmental surfaces and patients were not temporally or geographically related.

All *C. lusitaniae* isolates were evaluated by REA (29 isolates from seven patients, 2 environmental isolates, 2 control strains, and 1 ATCC strain). REA revealed a total of eight different restriction fragment length polymorphisms (strain types). Figure 1 shows the eight strain types from separate patients. Lanes A to F are the strains digested with *Msp*I. Because two groups had three isolates each, *Eco*RI digestion is shown for these six isolates (lanes I to N). Patients' locations reflecting predominant strain type are shown in Fig. 2. One strain was shared by five patients and the two environmental isolates. These patients were temporally and geographically related. One patient with this strain was moved between the ICU and the BMTU. One strain type was shared by a patient and control isolate. The isolates from the remaining patient, one control strain, and the ATCC strain were each found to be separate strains having unique restriction fragment length polymorphisms. Evaluation of isolates colonizing different body sites within an individual patient showed that four patients were colonized with the same strains at all sites. In three patients, two different strains of *C. lusitaniae* were identified in rectal and oropharyngeal cultures. Analysis of 17 isolates recovered from multiple sites in a single patient over a 12-week period showed persistence of the same strain. The restriction fragment length polymorphism pattern remained the same in all isolates and unique to that patient over the 12-week period of this patient's hospitalization. Also, the colonizing urine strain type was identical to the infecting strain type in the patient that developed the urinary tract infection with *C. lusitaniae*.

*Candida lusitaniae* is an infrequent opportunistic pathogen but of special interest in view of its ability to develop resistance to amphotericin B (1, 2, 8, 9, 15). All fatal cases of *C. lusitaniae* fungemia previously reported have involved resistant isolates (2, 3, 5, 8–10, 12, 13, 15, 16, 18, 21). Merz observed *C. lusitaniae* to be an infrequent isolate, representing 0.64% of yeast isolates recovered at the Johns Hopkins Hospital (13). Little is known about the risk factors for *C. lusitaniae* colonization and infection. Hadfield et al. (9) and Blinkhorn et al. (2) reported that of identified cases, most were immunocompromised patients with an underlying malignancy in the presence of prolonged broad-spectrum antibiotics, prolonged hospitalization, intravascular catheters, and cytotoxic or corticosteroid drugs. Nosocomial acquisition of *C. lusitaniae* has never been demonstrated.

We found *C. lusitaniae* was more common than anticipated, and it was cultured from 7% of patients and was 7.4% of all *Candida* specimens isolated. Case-control analysis

found no significant differences between patients with and without *C. lusitaniae* with regard to duration in the study unit, antibiotic use, antifungal prophylaxis, immunosuppressive or cytotoxic drug use, catheter use, hyperalimentation, or severity of underlying illness. The majority of patients in this study had serious underlying diseases, resulting in ICU or BMTU admission. These results are therefore similar to the trends noted by Blinkhorn et al. (2) and Hadfield et al. (9). Patients with *C. lusitaniae* colonization in this study were predominantly female and significantly older than controls, an observation not previously noted and of uncertain significance. In contrast to earlier studies, all isolates of *C. lusitaniae* were susceptible to amphotericin B, making this agent a therapeutic option. The reasons for these findings are not known. Nystatin is not used routinely in our units and may have an influence, considering that nystatin, a polyene antifungal agent, may increase the selection pressure for cross-resistance to amphotericin B.

Current understanding of the epidemiology of *Candida* species concludes that organisms are endogenously acquired from a patient's own flora (17). There have been occasional reports of *Candida* species outbreaks with suspected patient-to-patient spread or suspected acquisition from environmental sources (4, 11, 22, 24, 27). This study demonstrates exogenous acquisition of *C. lusitaniae* within the hospital environment and traces the route of acquisition. Nosocomial acquisition of *C. lusitaniae* was shown for five patients who were geographically and temporally associated in a BMTU and an ICU. All patients were cared for by the same unit personnel. We also found that individual patients carried more than one strain type. Patients, however, usually carried the same strains at multiple sites and over time. The mechanism of acquisition of *C. lusitaniae* remains uncertain. However, indirect contact transmission via carriage on the hands of hospital personnel is suggested by clustering of patients with identical strains and by the isolation of *C. lusitaniae* from an environmental surface frequently in contact with hands. The potential hospital reservoirs include colonized patients, hospital personnel, and the inanimate hospital environment. These findings show that nosocomial *C. lusitaniae* colonization occurred and was the result of exogenous acquisition of strains. Although the incidence of colonization is low and infection by *C. lusitaniae* is rare, the isolation of *C. lusitaniae* from the environment and patients indicates that the potential for spread exists.

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